REMARKS

Entry of the foregoing, reexamination and further favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. Section 1.112, , are respectfully requested.

By the present amendment, the specification has been amended to insert the specific section headings. Claim 31 has been amended to reflect that the polypeptide is purified and hence is not a natural polypeptide. Support for this amendment appears at least at page 29 of the specification as filed. Claim 38 has been added. Support for new claim 38 appears at least on page 14 of the specification. Applicants submit that no new matter has been added via this amendment.

Claims 31, 32 and 35-37 have been rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. For the following reasons, this rejection is respectfully traversed.

Applicants submit that the specification on at least pages 30 and 31 provides some guidance on how the T and B epitopes were recognized and that a person skilled in the art could easily identify B and T cell epitopes using known detailed procedures which were available prior to the filing of the present application. Thus, the Examiner's assertion that the specification does not specifically define the T epitope and B epitope is not correct.

Furthermore, although a "cook book" method for identifying T and B epitopes is not present in the specification, detailed procedures of how to identify T and B cell epitopes were well known in the art prior to the filing of the present application as evidenced by enclosed Annexes I, II and also *Longono et al.* of record. Thus, Applicants submit that it was not necessary to provide in detail in the specification that which was well known in the art. This fact is supported by the Federal Circuit in, for instance, *S3 Inc. v. VIDIA Corp.*, 259 F.3d 1364, 1371, 59 USPQ2d 1745 (Fed. Cir. 2001) where the court stated:

> The law is clear that patent documents need not include subject matter that is known in the field of the invention and is in the prior art, for patents are written for persons experienced in the field of the invention.

Furthermore, the legal issue which needs to be addressed with respect to this enablement rejection is whether the skilled artisan can practice Claims 31, 32 and 35-37 of record without undue experimentation. The legal criteria for evaluating the undue

experimentation issue are set forth in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988) and are the following:

(1) the state of the prior art;
(2) the relative skill of those in the art:
(3) the nature of the invention;
(4) the predictability or unpredictability of the art.
(5) the amount of direction or guidance presented;
(6) the presence or absence of working examples;
(7) the quantity of experimentation necessary; and
(8) the breadth of the claims.

It should be said that the state of the prior art with respect to the identification of B cell epitopes and T cell epitopes was well known. The identification of B epitopes and T epitopes was common practice at the time of the filing of the present invention as evidenced by the enclosed Annex I, which is an Immunology text book and enclosed Annex II, which are some articles which were available on this topic prior to the priority date of the present invention. Moreover, the specification at least on pages 30 and 31 provides a procedure in which the Applicants have identified the T and B epitopes.

It cannot be denied that the level of skill in the art with respect to the fields of Immunology, malaria and vaccine compositions to treat malaria was very high. This is evidenced by the prior art in this field at the time of filing of the present invention.

Identifying a purified polypeptide having a T or a B epitope or a vaccine composition containing same was not an unpredictable art, since the basic tools were available and in prior use.

The present specification provides an abundance of guidance and working examples on how to obtain purified polypeptides having B or T epitopes. Starting at least on page 25 in the specification is a detailed description on how to construct a genomic library from *P. falciparum*, how to immunologically screen the bank, how to select recombinant clones, how to complementary screen the subpopulation of clones, how to measure the size of the native protein of the LSA in the parasite, electron microscopy for confirmation of where the LSA is distributed in the hepatic schizonts and the immunological responses obtained in order to specify the biological function of the LSA protein.

Furthermore, the immunization of mice, the study of lymphocytes on subjects exposed to malaria, the identification of the T epitopes and B epitopes, as well as the identification of specific amino acid sequences containing these epitopes are also described in detail in the specification.

Although the breath of the claims are not limited, Applicants submit that the person skilled in the art can easily obtain other purified polypeptides having B or T epitopes from other *Plasmodium falciparums* by following the specification and the experimentation would only be routine and not undue.

Therefore, when evaluating undue experimentation according to the legal criteria in *In re Wands*, *supra*, Applicants submit that the specification, as well as the knowledge of those skilled in the art, provides enough guidance to obtain the T epitopes and B epitopes, as well as a vaccine composition containing same as set forth in the claims.

Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

Claims 31, 35 and 36 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3 of U.S. patent No. 6,319,502.

Applicants request that this rejection be held in abeyance until there is allowable subject matter. At that time, Applicants will file a Terminal disclaimer.

Claims 31, 35 and 36 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-6 of U.S. Patent No. 5,690,941. For the following reasons, this rejection is respectfully traversed.

U.S. Patent No. 5,690,941 relates to SALSA, which is a Sporozoite And Liver Stage Antigen, which contains T cell epitopes. Since this antigen is present at both sporozoite and liver stages, it cannot be considered as a liver stage specific antigen, as currently claimed in the present invention.

Further studies conducted by some of the inventors in order to verify the immunogenicity of *P. falciparum* pre-erythocyte antigens such as SALSA and LSA1 confirmed that LSA1 is really a liver specific antigen, while the SALSA antigen is not. Thus, for example, the publication submitted as Annex III of *Perlaza et al.*, *Infection and Immunity*, p. 3423-3428 (July 1998) at the sentence bridging pages 3425-3426, stated the following:

> The serum of *Aotus* monkey M21, immunized with LSA1 peptides, reacted specifically with liver schizonts but, as expected, not with other parasitic stages, thus confirming the strict liver stage expression of this molecule.

In conclusion the rejected claims clearly recite that the T epitope is from the liver-stage specific protein produced by *P. falciparumn*, which cannot be obvious in view of a protein present at both sporozoite and liver stages; i.e.. the SALSA protein.

Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

Claims 31, 35 and 36 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-3 and 7 of U.S. Patent No. 5,599,542. For the following reasons, this rejection is respectfully traversed.

U.S. Patent No. 5,599,542 discloses a purified polypeptide comprising an amino acid sequence which is the repeat sequence of 17 amino acids derived from the liver stage, which is rich in glutamine, glutamic acid and leucine. This same sequence is described in the present invention, but is not claimed. There is no suggestion in this patent that this sequence contains B or T epitopes. Nor does the sequence in U.S. Patent No. 5,599,542 contain B or T epitopes.

This is evidenced by the present patent specification at page 30 where it is explicitly stated that the T epitope for man and mouse is not defined by the repetitive part of the LSA.

Therefore Claims 31, 35 and 36 cannot be obvious in view of Claims 1-3 and 7 of U.S. Patent No. 5,599,542.

Claims 31, 35 and 36 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-3 of U.S. patent No. 6,270,771.

Applicants request that this rejection be held in abeyance until there is allowable subject matter. At that time, Applicants will file a Terminal Disclaimer

Claims 31, 32 and 35 to 37 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-16 and 25 of co-pending Application No. 09/900,963.

Applicants request that this rejection be held in abeyance until there is allowable subject matter for either application. At that time, Applicants will file a Terminal Disclaimer.

Claims 31, 32 and 35 have been rejected under 35 U.S.C. § 101 as being directed to non-statutory subject matter. These claims have been amended to recite "purified" which should render this rejection now moot. Accordingly, withdrawal of the rejection is respectfully requested.

Claims 31, 35 and 36 have been rejected under 35 U.S.C. § 102 (b) as being anticipated by or in the alternative under 35 U.S.C. § 103(a) as obvious over *Londono et al.* 1990 (*J. Immunol.* 145/5:1557-1563 (Abstract only) or *Guerin-Marchand et al.* 1987 (*Nature*, 329/6135:164-167). For the following reasons, however, this rejection is respectfully traversed.

Londono et al. describe two hybrid synthetic peptides, a di-component hybrid and a tri-component hybrid derived from two *Plasmodium falciparum* pre-erythrocyte antigens. More specifically, the di-component hybrid construct contained a circumsporzoite T cell epitope and an LSA repeat sequence, while the tri-component hybrid contained an additional sequence placed on the N-terminal of the di-component hybrid of a circumsporozoite repeat tetrapeptide of NPNA.

Londono et al. fail to disclose a purified polypeptide comprising at least one T epitope from a liver-stage specific protein produced by *P. falciparum*. Rather this reference describes a T cell epitope from the circumsporozoite stage, which is the initial stage of development of the parasite in man and corresponds to the sporozoite form introduced into the blood of the host by bites by insects that carry the parasite. This fact is clear from *Londono et al.* at page 1557, second column, first full paragraph where it is stated:

> In this context, the design of multivalent vaccines combining molecules of both sporozoite and liver stage sequences may constitute a logical complementary approach.

See also page 1 of the specification as filed and the Abstract of *Lonodo et al.* where it is stated that the hybrids contain a circumsporozoite T cell epitope.

Therefore, the *Lonodo et al.* fail to teach, either expressly or inherently, the presently claimed invention.

Guerin-Marchand et al. disclose a liver-stage specific antigen characterized by gene cloning. More specifically a clone DG307 contained 196 base pairs composed entirely of a 51-bp repeat sequence. A synthetic peptide having part of the repeat sequence: EQQSDLEQERLAKEKLQ was synthesized and its immunogenicity was examined. It was concluded that this 17 amino acid repeat carries at least one epitope corresponding to an antibody in human sera.

Guerin-Marchand et al. fail to disclose a purified polypeptide comprising at least one epitope from a liver-stage protein produced by *P. falciparum* or additionally containing a B epitope. Neither T nor B epitopes are present on the antigens described in this reference. This should be clear from the present specification which states at least on page 30 that: "a T epitope for man and mouse is not defined by the repetitive part of the LSA molecule."

Therefore, Applicants submit that *Guerin-Marchand et al.* do not teach, either expressly or inherently, the claimed invention.

As far as the obviousness portion of the rejection is concerned, *Londono et al.* specifically describe at page 1558, first column, the following:

> Previous studies using mice and synthetic as well as recombinant peptides showed that the LSA structure was unable to prime or boost either specific antibody responses *in vivo* or T cell proliferation *in vitro*.

Thus, this teaching would lead a skilled artisan away from seeking a T epitope in the LSA structure. It is well settled in the case law that references that teach away from the present invention cannot serve as a basis for establishing a *prima facie* case of obviousness. *McGinley v. Franklin Sports, Inc.* 262 F.3d 1339, 60 U.S.P.Q.2d 1001 (Fed. Cir. 2001).

Therefore, Applicants submit that the presently claimed invention is unobvious in view of *Londono et al.*

Guerin-Marchand et al. disclose liver stage-specific antigens of *P. falciparum* which were characterized by gene cloning. This reference only described a repeated sequence, which, as set forth above, does not contain T epitopes nor B epitopes. Furthermore, *Guerin-Marchand et al.* do not provide any guidance of how to obtain antigens with B and T epitopes nor provide any suggestion of the possibility that antigens having B and T epitopes are contained within the LSA structure.

As stated in *Sibia Neurosciences Inc. v. Cadus Pharmaceutical Corp.*, 55 U.S.P.Q.2d 1927 (Fed. Cir. 2000):

> To establish a prima facie case of obviousness, 'the prior art reference (or references when combined) must teach or suggest all the claim limitations MPEP § 2142.

Since *Guerin-Marchand* is silent with respect to obtaining purified polypeptides that comprise at least one T epitope from a liver-stage specific protein produced by *P. falciparum*, Applicants submit that this obviousness rejection cannot be maintained.

Thus, in view of the above, withdrawal of this rejection is respectfully requested.

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: March 12, 2004

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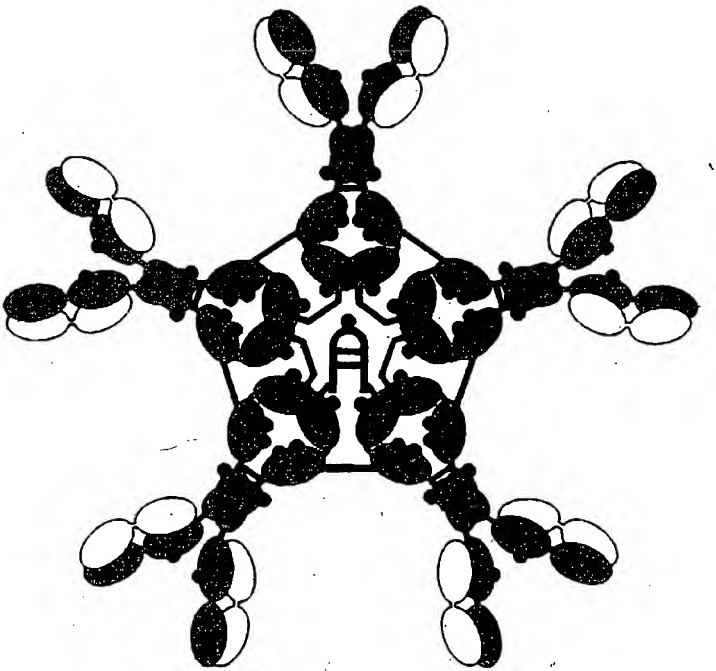
ANNEX I

ANNEX I

IMMUNOLOGY

Ivan Roitt · Jonathan Brostoff · David Male

SECOND EDITION



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MARKERS

Lymphocytes and other leucocytes express a large number of different molecules on their surfaces. Some of these appear at particular stages of cell differentiation or activation for short periods, while others are characteristic of different cell lineages. Such molecules which can be used to distinguish cell populations are called markers, and many of them can be identified by specific monoclonal antibodies. Recently a systematic nomenclature has been developed for these cell surface molecules – the CD system, in which the markers are numbered CD1, CD2, etc. The term CD (cluster designation) was derived by computer analysis of monoclonal antibodies raised in different laboratories worldwide against human leucocyte antigens. An international workshop determined their patterns of staining on leucocytes and the weights of the molecules precipitated by the antibodies. Monoclonal antibodies with similar specificity characteristics were grouped together and given a CD number. This number is now also used to indicate the specific molecule recognized by a group of monoclonal antibodies (see Fig. 2.11). In many cases the functions of the molecules are known, for example the marker CD35 is a complement C3b receptor (CR1).

Markers may be demonstrated using fluorescent antibodies as probes. In this case the surface markers act as antigens (Fig. 2.7). Hybridoma technology for making these antibodies, together with flow cytometry techniques, which allows enumeration and the separation of cells on the basis of their size and fluorescence intensity (see Chapter 25) has revolutionized studies on the functional activities of lymphoid cell populations.

T CELLS

One of the first ways of distinguishing human T cells from B cells was by their ability to bind to sheep erythrocytes which is through the CD2 molecule (Fig. 2.8). However the definitive T cell marker is the T cell antigen

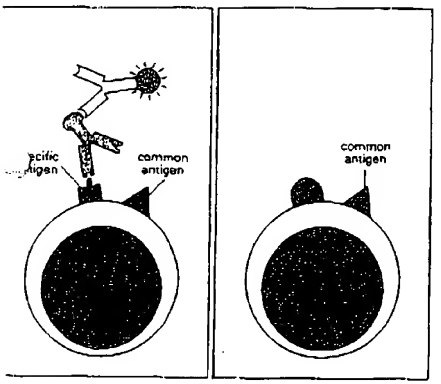

Fig. 2.7 Immunofluorescent method for the demonstration of T cell markers. Mouse antibodies directed towards T cell subset-specific antigen on a cytotoxic or suppressor T cell (Tc/s) will bind to this antigen but not to the T cell-specific antigen common to the T-helper (TH) subset. The bound antibody is detected using antibodies to mouse immunoglobulin coupled to a fluorescent molecule.

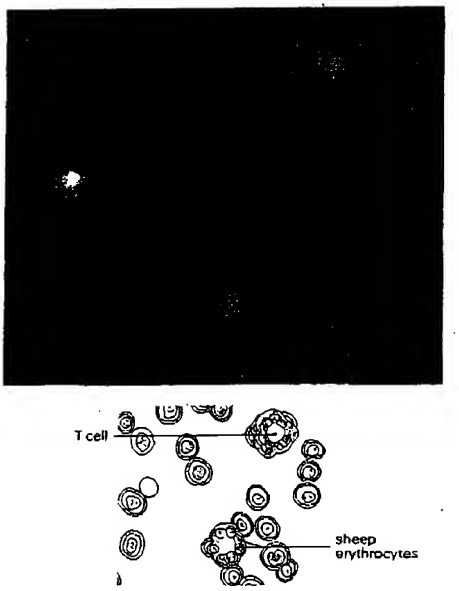

Fig. 2.8 Typical marker of human T cells. Human T cells from blood and tissues have the fortuitous property of binding to sheep erythrocytes (SE). Following their centrifugation together, T cells are distinguishable by their ability to form rosettes with SE. The nucleated cells are distinguished from the SE by green fluorescent staining of the nuclei and cytoplasm with acridine orange. The formation of rosettes with SE by T cells also provides a means for the physical separation of T from non-T cells (see Chapter 25).

receptor (TCR). There are presently two defined types of TCR; TCR-2 is a heterodimer of 2 disulphide-linked polypeptides (α and β), TCR-1 is structurally similar but consists of γ and δ polypeptides. Both receptors are associated with a complex of polypeptides making up the CD3 complex (see Chapter 5). Thus a T cell is defined either by TCR-1 or TCR-2 which is associated with CD3.

Approximately 95% of blood T cells express TCR-2 and up to 5% have TCR-1. The TCR-2 bearing cells can be subdivided further into two distinct non-overlapping populations; the T_H subset which is $CD4^+$ and the T_{cs} subset which is $CD8^+$. $CD4^+$ T cells recognize antigens in association with major histocompatibility complex (MHC) class II molecules (see Chapter 4), while $CD8^+$ T cells recognize antigens in association with MHC class I molecules.

The $CD4^+$ set can be further divided functionally into:

1. those cells which positively influence the immune response of T cells and B cells – the helper cell function, which are $CDw29^+$
2. cells inducing suppressor/cytotoxic functions in $CD8^+$ cells – the suppressor inducer function, which are themselves $CD45R^+$.

Other monoclonal antibodies and criteria have been used to subdivide the $CD4^+$ set. For example, all $CD4^+$

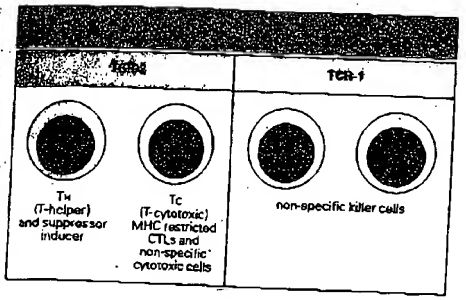

Fig. 2.9 T cell lineages. T cells may be differentiated according to their expression of the antigen receptors TCR-1 or TCR-2 and expression of surface molecules CD4 or CD8. These correspond with the functional categories of cells.

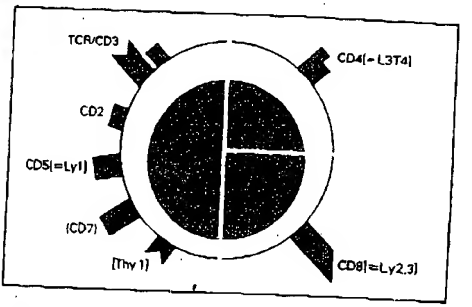

Fig. 2.10 Major T cell markers in man and mouse. The molecule CD7 is bracketed to indicate that it is only detected thus far in man. Markers in square brackets are specific for mouse (Thy-1) or mouse equivalents.

CD1a	49	thymocytes, Langerhans' cells	
CD1b	46	thymocytes	
CD1c	43	thymocytes	
CD2	50	T cells	sheep erythrocyte receptor
CD3	20—26	T cells	part of T cell antigen receptor complex
CD4	60	T cell subset T_H	MHC class II restricted immune recognition
CD6	67	T cells, B cell subset	
CD8	120	T cells	
CD7	40	T cells	
CD8	32	T cell subset (Tc/s)	? IgM Fc receptor
CD10	100	pre B cells	MHC class I restricted immune recognition
CD11a	180	leucocytes	α chain of LFA-1 (adhesion molecule)
CD11b	160	monocytes, granulocytes	α chain of complement receptor CR3 (MAC-1)
CD11c	150	monocytes, granulocytes	α chain of p150, 95 (complement receptor/adhesion molecule)
CD13	150	granulocytes, monocytes	
CD14	55	monocytes, (granulocytes)	
CD15	50—180	granulocytes, monocytes	carbohydrate hapten
CD16	50—60	granulocytes (macrophages)	Fc receptor ($Fc_\gamma R$ III) low affinity
CD17		granulocytes, monocytes, platelets	
CD18	95	leucocytes	β chain of LFA-1, CR3 and p150, 95
CD19	95	B cells	
CD20	35	B cells, dendritic cells	
CD21	140	B cells, dendritic cells	complement receptor CR2
CD22	135	B cells	
CD23	45	activated B cells	IgE receptor (low affinity)
CD25	55	activated T cells, B cells and macrophages	IL-2 receptor (low affinity polypeptide); α chain
CD28	44	T cell subset	
CDw29	135	T cell subset and many non-leucocytes	
CDw32	40	monocytes, granulocytes, platelets, B cells	Fc receptor ($Fc_\gamma R$ II)
CD33	67	granulocyte/monocyte precursors, monocytes	
CD34	115	granulocyte/monocyte precursors	
CD35	220	B cells, erythrocytes, granulocytes, dendritic cells, T cells, plasma cells	complement receptor CR1
CD38	45	plasma cells, T cells	
CDw41	140 + 95	megakaryocyte, platelets	Gp11b/111a
CDw42	150	megakaryocytes, platelets	Gp1b
CD45	180—220	leucocytes	leucocyte common antigen
CD45R	220, 205	B cells, T cell subsets, granulocytes, monocytes	restricted leucocyte common antigen

Fig. 2.11 Summary of the main CD molecules. This table shows some of the CD molecules characterized so far. The majority do not show absolute lineage specificity, but some are more lineage related than others.

cells expressing natural killer (NK) cell markers produce the lymphokine interleukin-2 (IL-2), and do not proliferate in response to antigens and mitogens. In fact, recent *in vitro* studies on $CD4^+$ clones in mouse and man have defined two separate populations (TH1 and TH2) based on the production of different lymphokines.

$CD8^-$ T cells can also be subdivided by a number of criteria and a variety of monoclonal antibodies into specific functional subsets. For example cells which recognize antigen in association with MHC molecules and produce IL-2 ($CD28^+$) and cells which do not recognize antigen in association with MHC molecules or produce IL-2 ($CD11b^+$).

$CD3^+$/TCR-1$^+$ cells represent a minority of circulating T cells which are also $CD4^-$, $CD8^-$. These cells home in to surface epithelia such as the epidermis and mucosal epithelia and are termed intra-epithelial lymphocytes (IEL). In interstitial mucosal epithelium TCR-1$^+$ cells also express CD8. It is probable that these cells represent a primitive cytotoxic population operating at the sites of entry of pathogens. Division of the TCR, CD4 and CD8 bearing cells into different functional subsets is illustrated in Figs 2.9 and 2.10.

Thus far we have described the antigen-specific receptors and markers allowing definition of T cell subsets. There are also a number of other surface molecules, expressed on all T cells (pan T cell markers) which are also found on cells of other lineages. For example, as shown above, all T cells express receptors for sheep erythrocytes (CD2). This molecule together with the TCR/CD3 complex and other membrane bound glycoproteins is involved in activating T cells when recognized by the appropriate ligand. CD2 is also found on about 50% of $CD3^-$ NK cells. CD5 molecules are expressed on all T cells, and also on a subpopulation of B cells which are involved in autoantibody production. CD7 is seen on all third population cells, and this molecule may be the receptor for the Fc portion of IgM. A summary of the more important CD markers is given in Fig. 2.11, which includes those seen on T cells and those present on other haemopoietic cells.

Murine T cells express markers similar to those detected on human T cells (see Fig. 2.10). In addition, all murine T cells carry a molecule, Thy-1 or θ, with a molecular weight of 19–35 kD. With regard to suppressor cells in the mouse, a small proportion of T cells carry I-J molecules, the expression of which is controlled by genes in the MHC (see Chapter 10). The binding of the lectin *Vicia villosa* might also mark a population in man and mouse which is involved in 'contrasuppression'.

B CELLS

B lymphocytes represent about 5–15% of the circulating lymphoid pool and are classically defined by the presence of endogenously produced immunoglobulins (antibody). These molecules are inserted into the surface membrane where they act as specific antigen receptors. They are detected on the surface of mature cells by staining cell suspensions with fluorochrome-labelled specific antibodies to the appropriate immunoglobulin of the species under investigation. Staining of cells in the cold results in the detection of the fluorescence with a 'ring-like' (or patchy) appearance over the cell (Fig. 2.12).

The majority of human peripheral blood B lymphocytes express both surface IgM and IgD molecules, which share the same specificity on the same cell. Very few

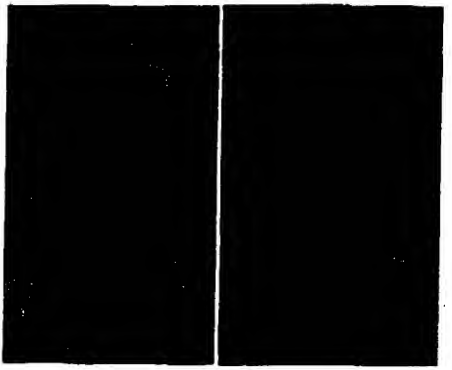

Fig. 2.12 B cells stained for surface immunoglobulin. Human blood B cells stained in the cold with fluoresceinated anti-human immunoglobulin show a patchy surface fluorescence viewed under ultraviolet light (right). Under phase contrast light microscopy (left) it can be seen that only 2 out of the 6 cells in this field are B lymphocytes. The lower cell shows 'capping' of the fluorescent antibody.

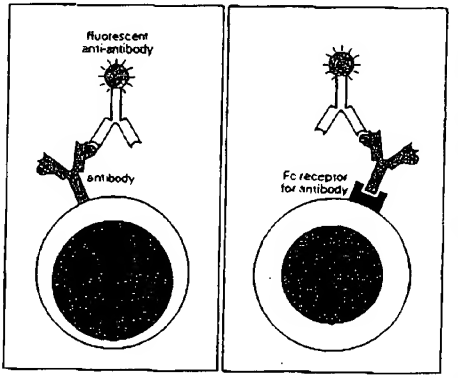

Fig. 2.13 Visualization of antibody bound to B cells and non-B cells by immunofluorescence. The surface antibody on the B cell is detected using a fluorescein anti-antibody conjugate (left). This conjugate will also 'detect' non-B cells carrying receptors for the Fc part of the antibody and which have antibody on their surface (right).

cells express surface IgG, IgA or IgE in the circulation although these are present in larger numbers in specific locations in the body, for example, IgA-bearing cells in the intestinal mucosa. Since other cells, in addition to B cells also carry surface receptors which non-specifically bind to antibodies, care should be taken in evaluating the number of B cells when using anti-immunoglobulin reagents. Antibodies bound to these receptors (Fc receptors) can also stain with fluoresceinated anti-human immunoglobulin (Fig. 2.13).

Divalent antibodies will cross-link antigens on surface

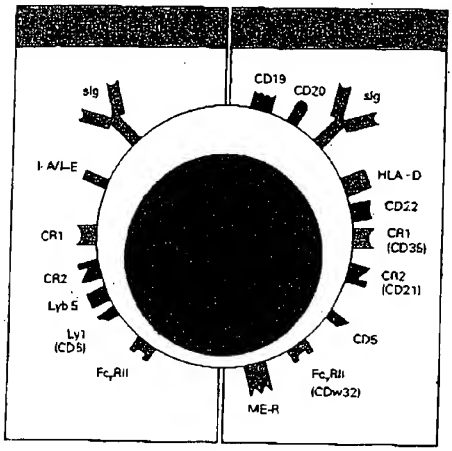

Fig. 2.14 Summary of the surface markers on mouse and human peripheral B cells. Equivalent molecules are in the same colour.

CD16	minority of T cells, granulocytes, some macrophages
CD11b	granulocytes, monocytes, some T cells
CD38*	activated T cells, plasma cells, haemopoietic precursors
CD2*	all T cells
CD7	all T cells
CD8*	some T cells
Leu19,NHK-1 (270 kD)	some T cells
Leu7* (110 kD)	some T cells
IL-2R (β chain, p70)	activated T cells

*Expressed on 10–80% of TPCs only.

Fig. 2.15 Surface markers of human TPCs.

Thy 1*	T cells
Lyb5 (T200)	B cells
NK1	–
NK2	–
FcγR*	some T cells, granulocytes some monocytes/macrophages
Asialo GM1	–
Cla (CD11b, MAC-1)	granulocytes, monocytes

*Expressed on some but not all murine TPCs.

Fig. 2.16 Surface markers of mouse TPCs.

membrane glycoproteins and be seen as 'patches' of cross-linked antigen–antibody complexes on the cell surface. Most of these complexes are actively swept along the cell surface and are seen as a 'cap' over one pole of the cell (see Fig. 2.12, right). This phenomenon is not peculiar to immunoglobulin on B cells but may also be seen with surface glycoproteins on other cell types when multivalent antibodies are attached to them.

A number of other markers are carried by both mouse and human B cells but not by resting T cells (Fig. 2.14). B cells are defined as those cells carrying endogenously produced immunoglobulins. The majority of B cells carry MHC class II antigens which are important in cooperation with T cells. These are I-A/I–E in the mouse and HLA–DP, DQ and DR antigens in man. Complement receptors for C3b (CR1, CD35) and C3d (CR2, CD21) are commonly found on B cells and are associated with activation and possibly homing of the cells. In this regard, the CR2 molecule is also the receptor for the Epstein-Barr virus (EBV) which on entry leads to the activation of the B cell. Fc receptors for IgG (FcRII, CDw32) are also present. CD19, CD20 and CD22 are the main markers currently used to identify human B cells. A marker originally found only on T cells (Ly1, CD5) has now been shown to be present on some B cells and identify a subset which is predisposed to autoantibody production. Some human B cells bind to mouse erythrocytes. The mouse erythrocyte rosette (ME-R), together with CD5, probably identifies an immature B cell population and both markers have been useful in the diagnosis of human lymphoproliferative disorders. Lyb5(T200) is a mouse alloantigen found on all B cells.

THIRD POPULATION CELLS

Third population cells (TPCs) have already been defined by morphology as large granular lymphocytes (see Fig. 2.2). TPCs possess larger numbers of electron-dense granules than granular T cells. They account for up to 20% of blood lymphocytes and can be negatively defined as cells lacking conventional surface antigen receptors, that is, TCR or immunoglobulin. Most surface antigens detectable on TPCs by monoclonal antibodies are shared with T cells or cells of the myelomonocytic series. The major markers of human TPCs and their shared specificities are shown in Fig. 2.15. A reagent commonly used to identify TPCs in purified lymphocyte populations is the monoclonal antibody to CD16 ($Fc_\gamma R$ III, $Fc_\gamma R$III). This is also expressed by a small proportion of T cells, by granulocytes and by some macrophages. Resting TPCs also express the α chain of the IL-2 receptor, an intermediate affinity receptor of 70 kD. Therefore, direct stimulation with IL-2 results in activation of TPCs. The

ANNEX II

Annex II

http://www.epitope-informatics.com/References.htm

B cell epitope prediction reviews

Stern, P.S. (1991). Predicting antigenic sites on proteins. *Trends in Biotechnology*, 9: 163-169.

Pellequer, J.L., Westhof, E. and Van Regenmortel, M.H.V. (1991). Predicting location of continuous epitopes in proteins from their primary structures. *Methods in Enzymology*, 203: 176-201.

Laver, W.G., Air, G.M., Webster, R.G. and Smith-Gill, S.J. (1990). Epitopes on protein antigens: misconceptions and realities. *Cell*, 61: 553-556.

Hopp, T.P. (1989). Use of hydrophilicity plotting procedures to identify protein antigenic segments and other interaction sites. *Methods in Enzymology*, 178: 571-585.

Van Regenmortel, M.H.V. (1989). The concept and operational definition of protein epitopes. *Philosophical Transactions of the Royal Society of London*, 323: 451-466.

Getzoff, E.D., Tainer, J.A., Lerner, R.A. and Geysen, H.M. (1988). The chemistry and mechanism of antibody binding to protein antigens. *Advances in Immunology*, 43: 1-98.

Van Regenmortel, M.H.V. and de Marcillac, G.D. (1988). An assessment of prediction methods for locating continuous epitopes in proteins. *Immunology Letters*, 17: 95-108.

Hopp, T.P. (1986). Protein surface analysis: methods for identifying antigenic determinants and other interaction sites. *Journal of Immunological Methods*, 88: 1-18.

Van Regenmortel, M.H.V. (1986). Which structural features determine protein antigenicity? *Trends in Biochemical Sciences*, 11: 36-39.

Other B cell epitope prediction

Hofmann, H-J., Hädge, D., Höltje, M. and Höltje, H-D. (1991). Theoretical prediction of antigenic determinants based on protein-water interaction energies. *Quantitative Structure-Activity Relationships*, 10: 300-305.

Hofmann, H-J., Hädge, D., Höltje, M. and Höltje, H-D. (1990). Protein-water interaction energies as predictor for antigenic determinants. *Molecular Immunology*, 27: 1057-1060.

Krchňák, V., Mach, O. and Malý, A. (1989). Computer prediction of B-cell determinants from protein amino acid sequences based on incidence of β turns. *Methods in Enzymology*, 178: 586-611.

Stern, P.S. (1989). Normal-mode dynamics as a tool for predicting antigenic sites on proteins. *Progress in Clinical Biological Research*, 289: 87-94.

Jameson, B.A. and Wolf, H. (1988). The antigenic index: a novel algorithm for predicting antigenic determinants. *Bioinformatics*, 4: 181-186.

Cornette, J.L., Cease, K.B., Margalit, H., Spouge, J.L., Berzofsky, J.A. and DeLisi, C. (1987). Hydrophobicity scales and computational techniques for detecting amphipathic structures in proteins. *Journal of Molecular Biology*, 195: 659-68

T cell epitopes and prediction

Reyes, V.E., Lew, R.A., Lu, S. and Humphreys, R.E. (1991). Prediction of α helices and T cell-presented sequences in proteins with algorithms based on strip-of-helix hydrophobicity index. *Methods in Enzymology*, 202: 225-238.

Menédez-Arias, L. and Rodríguez, R. (1990). A BASIC microcomputer program for prediction *of B and T cell epitopes in proteins. Bioinformatics, 6: 101-105.*

Cornette, J.L., Margalit, H., DeLisi, C. and Berzofsky, J.A. (1989). Identification of T-cell epitopes and use in construction of synthetic vaccines. *Methods in Enzymology*, 178: 611-634.

Stille, C.J., Thomas, L.J., Reyes, V.E. and Humphreys, R.E. (1987). Hydrophobic strip-of-helix algorithm for selection of T cell-presented peptides. *Molecular Immunology*, 24: 1021-1027.

1: J Immunol. 1987 Apr 1;138(7):2213-29.

Related Articles

Prediction of immunodominant helper T cell antigenic sites from the primary sequence.

Margalit H, Spouge JL, Cornette JL, Cease KB, Delisi C, Berzofsky JA.

We have used a data base of 23 known immunodominant helper T cell antigenic sites located on 12 proteins to systematically develop an optimized algorithm for predicting antigenic sites. The algorithm is based on the amphipathic helix model in which antige sites are postulated to be helices with one face predominantly polar and the opposite fa predominantly apolar. Such amphipathic structures can form when the polarity of resid along the sequence varies with a more or less regular period. Hence they can be identif by methods (so called power spectrum procedures) that detect periodic variations in properties of a sequence. The choice of power spectrum procedure, hydrophobicity sca and model parameters are examined. An algorithm is tested by comparing the predicte amphipathic segments with the locations of the known T cell sites, counting the numbe matches, and calculating the probability of getting this number by chance alone. The optimum algorithm, which predicts the largest number of sites with the lowest chance probability, uses the Fauchere-Pliska hydrophobicity scale and a least squares fit of a sinusoid as its power spectrum procedure. By applying this algorithm, 18 of the 23 kno sites are identified (75% sensitivity) with a high degree of significance (p less than 0.0 The success of the algorithm supports the hypothesis that stable amphipathic helices ar fundamentally important in determining immunodominance. This approach may be of practical value in designing synthetic vaccines aimed at T cell immunity.

PMID: 2435793 [PubMed - indexed for MEDLINE]

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ANNUAL REVIEWS

The structure of T-cell epitopes.

Livingstone AM, Fathman CG.

We have reviewed here studies using synthetic peptides to analyze some of the propert T-cell epitopes. Several general conclusions can be drawn. First, T-cell epitopes can us be defined by linear sequences of about seven amino acids. However, the observation t increasing peptide length often results in increased antigenic potency has suggested tha antigenicity may crucially depend upon the ability of peptides to adopt appropriate secondary structures. Two models for the prediction of T-cell epitopes on the basis of primary sequence data alone were discussed. Biophysical studies on the association of peptides with Ia molecules have shown that antigenic peptides bind directly to Ia; the evidence suggests that a binary association between Ia and peptide occurs in the absen specific T-cells. Finally, a hypothesis to explain the observation that B-cells and T-cell generally recognize distinct epitopes on multideterminant antigens has been examined.

Publication Types:
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Related Articles

Strong conformational propensities enhance T cell antigenicity.

Spouge JL, Guy HR, Cornette JL, Margalit H, Cease K, Berzofsky JA, DeLisi C.

The ability to predict T cell antigenic peptides would have important implications for tl development of artificial vaccines. As a first step towards prediction, this report uses a statistical technique to discover and evaluate peptide properties correlating with T cell antigenicity. This technique employs Monte Carlo computer experiments and is applic: to many problems involving protein or DNA. The technique is used to evaluate the contribution of various peptide properties to helper T cell antigenicity. The properties investigated include amphipathicities (alpha and beta), conformational propensities (al beta, turn and coil), and the correlates of alpha-helices, such as the absence of helix-bre and the positioning of the residues which stabilize alpha-helical dipoles. We also inves segmental amphipathicity. (A peptide has this property when it contains at least two di: subpeptides, one hydrophobic, one hydrophilic.) Statistical correlations and stratificatic assessed independent contributions to T cell antigenicity. The findings presented here l important implications for the manufacture of peptide vaccines. These implications are follows: if possible, peptide vaccines should probably be those protein segments which a propensity to form amphipathic alpha-helices, which do not have regions with a prop to coil conformations, and which have a lysine at their COOH-terminus. The last two observations are of particular use in manufacturing peptides vaccines: they indicate wh the synthetic peptides should be terminated. These implications are supported by the findings given below. The significances (p values) support the following statistical generalites about antigenic conformations: most helper T cell antigenic sites are amphi alpha-helices; alpha-helical amphipathicity and propensity to an alpha-helical conform contribute independently to T cell antigenicity; there is evidence that some T cell antig sites are beta conformations instead of alpha-helices; T cell antigenic sites avoid rando coiled conformations; and T cell antigenic sites are usually not segmentally amphipath alpha-Helical amphipathicity was significant, but segmental amphipathicity was not. T has implications for the dimensions of the structure interacting with the hydrophobic p of an amphipathic T cell antigenic site. Lysines are unusually frequent at the COOH-terminal of T cell antigenic sites, even after accounting for tryptic digests. These lysine stabilize alpha-helical peptides by a favorable interaction with alpha-helical dipoles. (ABSTRACT TRUNCATED AT 400 WORDS)

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1: Vaccine. 1990 Feb;8(1):35-40. Related Articles

Polyvalent synthetic vaccines: relationship between T epitopes and immunogenicity.

Jolivet M, Lise L, Gras-Masse H, Tartar A, Audibert F, Chedid L.

Department of Immunology and Microbiology, University of South Florida College of Medicine, Tampa 33612-4799.

Three different synthetic polyvalent vaccines have been constructed by conjugating fou synthetic peptides without any carrier protein. The peptides were copy fragments of tw bacterial antigens (Streptococcus pyogenes M protein and diphtheria toxin), two parasi antigens (circumsporozoite protein of Plasmodium falciparum and Plasmodium knowle and one viral antigen (hepatitis B surface antigen). Outbred guinea-pigs immunized wi polyvalent vaccine containing streptococcal, diphtheric, P. knowlesi and hepatitis pepti raised high specific antibody response against the four specificities. Individual T cell analysis demonstrated that hepatitis peptide bears T dominant epitope. A similar immu response was obtained with a second polyvalent vaccine where the P. knowlesi peptide been replaced by the P. falciparum peptide. In both experiments the malarial peptides behave like pure B epitopes. Prediction of immunodominant helper T-cell antigenic sit were performed with the five peptides using computer algorithm. Hepatitis and diphthe peptides were selected whereas the streptococcal peptide was rejected although it can experimentally contain a T epitope. To confirm this result animals were immunized wi third polyvalent vaccine which does not contain the hepatitis peptide. No T cell prolife or antipeptide antibodies were detected. These results demonstrate that the cooperative immune response requires a certain degree of antigenic complexity for the induction of antibody response.

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1: Curr Top Microbiol Immunol. 1990;155:143-52. Related Articles

Prediction and identification of bacterial and parasitic T-cell antigen and determinants.

Rothbard JB, Lamb JR.

Molecular Immunology Laboratory, Imperial Cancer Research Fund, London, UK.

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Cloning of microbial epitopes relevant for T- and B-cells.

Miles MA, Wallace GR.

Department of Medical Parasitology, London School of Hygiene and Tropical Medicin U.K.

This review summarises, and illustrates, the technology that is available for the molecu cloning and precise identification of T-cell and B-cell epitopes, particularly those of ba and parasites. Methods include: selective cloning following subtractive hybridization o nucleic acids; selective screening of expression libraries; analysis of subcloned "epitop libraries" or "deletion constructs"; scanning of multiple synthetic peptides, and comput enhanced prediction. The direct sequencing of polymerase chain reaction products allo the rapid analysis of epitope heterogeneity occurring among natural populations. Multi epitopes can be assembled either by synthesis or by the expression of polymeric epitop bearing peptides. Prospects for probing expression libraries with T-cells are bleak due complexities of antigen processing, presentation and T-cell recognition in vitro. Elucid of the enzymic steps involved in processing, resolution of peptide/MHC II co-crystals, pairing of a large number of known epitopes with their functional restriction elements significantly improve the ability to predict T-cell epitopes.

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1: Nature. 1991 Feb 21;349(6311):720-1.

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nature

Identifying antigenic T-cell sites.

Feller DC, de la Cruz VF.

MedImmune, Inc., Gaithersburg, Maryland 20878.

Computer algorithms that have been used successfully on protein sequences for the prediction of antigenic T-cell sites have been collected into a single computer software package called TSites.

PMID: 1705016 [PubMed - indexed for MEDLINE]

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1: Eur J Immunol. 1989 Jan;19(1):43-7.

Efficient mapping and characterization of a T cell epitope by the simultaneous synthesis of multiple peptides.

Van der Zee R, Van Eden W, Meloen RH, Noordzij A, Van Embden JD.

Laboratory of Bacteriology, National Institute of Public Health, Bilthoven, The Nether

Prediction, identification and analysis of T cell epitopes in protein antigens has become central theme in fundamental and applied immunology. However, while for the characterization of linear B cell epitopes the so-called Pepscan procedure was found to extremely effective, no such technique has so far been available for T cell studies. Rec we described the identification and localization of a T cell epitope in a mycobacterial 6 kDa shock protein in the model of adjuvant arthritis. This was done by molecular cloni and conventional solid-phase synthesis techniques. We now show that the delineation c such a T cell epitope and its further characterization can be accomplished in a much m rapid and efficient manner by a modification of the existing Pepscan technique. We sh for the first time that several hundreds of peptides, simultaneously synthesized in an automated way on activated polyethylene rods, can be easily recovered from these rod adequate quantities, enabling a systematic analysis of T cell epitopes. Synthesis of sequentially overlapping peptides along the 65-kDa protein revealed that the adjuvant arthritis T cell clones are fully stimulated by peptides that comprise a minimal sequenc seven residues, corresponding to positions 180-186 in the sequence of the 65-kDa prot M. bovis Bacillus Calmette Guerin (BCG). Detailed examination of the epitope by pep containing a single amino acid substitution showed that, apart from one conservative replacement (Glu—Asp), the requirement for the native residue at all positions in pep 180-186 was absolute for full T cell stimulation. Their indispensability was confirmed deletion and insertion peptides. It is concluded that the occurrence of indifferent or spa residues in a minimal stimulatory sequence, as observed by others, is not a general feat T cell epitopes.

PMID: 2465904 [PubMed - indexed for MEDLINE]

1: J Exp Med. 1991 Nov 1;174(5):1059-71.

On the nature of peptides involved in T cell alloreactivity.

Rotzschke O, Falk K, Faath S, Rammensee HG.

Max-Planck-Institut fur Biologie, Abteilung Immungenetik, Tubingen, Germany.

The strong reaction of T cells against foreign major histocompatibility complex (MHC antigens, commonly termed "alloreactivity", is not only a nuisance for clinical organ transplantation; it also remains a puzzling question for immunologists. By making use recent technical developments, alloreactive T cells nominally directed against a mutati a single MHC class I molecule were found to fall into several major categories. One is recognizing peptides whose occurrence is dependent on one particular MHC allele, and is recognizing peptides supported by several MHC alleles, and a third is recognizing peptides occurring independently of MHC alleles. In a fourth category, the binding to ? of any of a broad range of peptides appears sufficient. In addition, there are T cells for which no peptide involvement could be detected at all. Even within these categories, th heterogeneity of T cells is considerable: among 16 Kb-reactive T cells analyzed, 15 dif modes of reactions were found.

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1: Eur J Immunol. 1991 Nov;21(11):2891-4. Related Articles

Exact prediction of a natural T cell epitope.

Rotzschke O, Falk K, Stevanovic S, Jung G, Walden P, Rammensee HG.

Max-Planck-Institut fur Biologie, Abteilung Immunogenetik, Tubingen, FRG.

T lymphocytes recognize their antigen as peptides associated with major histocompatibl complex (MHC) molecules. Peptides naturally presented by MHC class I molecules ar uniform in length and have a specific motif, both defined by the respective MHC allele (Falk, K. et al. Nature 1991. 351:290). These allele-specific motifs should allow exact prediction of natural T cell epitopes. H-2Kb-restricted epitopes, for example, have a le of eight amino acid residues and conserved anchor residues at positions 5 and 8. Accor to this information, we predicted the natural Kb-restricted epitope of ovalbumin, thoug be contained in the 19-mer IINFEKLTEWTSSNVMEER, to be SIINFEKL. Here we s that this prediction is correct. Thus, exact prediction of natural T cell epitopes is possib

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ANNEX III

Annex III

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Immunogenicity of Four *Plasmodium falciparum* Preerythrocytic Antigens in *Aotus lemurinus* Monkeys

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***Aotus lemurinus* monkeys were immunized with pools of either lipid-tailed peptides injected in PBS or peptides in Montanide ISA-51, all derived from four *Plasmodium falciparum* pre-erythrocytic antigens, namely, LSA1, LSA3, SALSA, and STARP. These formulations were well tolerated. Their immunogenicity was demonstrated by the induction of both B- and T-cell responses to most of the peptides studied (of the 12, 10 induced antibody production, 9 induced T-cell proliferative responses, and all 12 induced gamma interferon secretion). Immune responses proved to be long lasting, since some were still detectable 210 days after immunization. Of particular importance is the fact that B- and T-cell responses elicited in this way by synthetic peptides were specific for native parasite proteins on *P. falciparum* sporozoites and liver stage parasites.**

The possibility of developing malaria vaccines based on pre-erythrocytic antigens was first considered following the observation that immunization with X-radiation-attenuated sporozoites could induce protective immunity (17, 30). However, more recent studies carried out in parallel under in vivo and in vitro conditions have shown in both humans and rodents that protection depends on the abilities of irradiated sporozoites to penetrate hepatocytes and, further, to transform into uninucleate liver trophozoites (14). The indication that persistent liver form parasites are required to induce protection (14, 38, 46) was confirmed recently (34, 44).

Based on this rationale, we have focused our recent work on the identification and characterization of liver stage antigens (24, 35). Four of them, namely, LSA1, LSA3, SALSA, and STARP, were recently characterized (4, 12a, 21, 22). The B- and T-cell antigenicity of several regions of these four molecules was established by epidemiological studies (3a, 4, 12a, 21, 22), and the corresponding synthetic peptides were produced to study their immunogenicity.

Taking into account, on the one hand, the known potential of *Aotus lemurinus* as a model for erythrocytic stages of *Plasmodium falciparum* malaria (8, 10) and, on the other hand, the susceptibility of monkeys in the family *Cebidae* to *P. falciparum* liver stage development (11, 12, 13a, 14, 16), the aim of the present study was to gather preliminary indications about their capacity to develop an immune response to these antigens compared to mice, chimpanzees, and humans before embarking on systematic studies involving larger numbers of monkeys.

Immunization. Four *A. lemurinus griseimembra* monkeys (from northern Colombia) with karyotype II or III were enrolled in immunization experiments using 12 synthetic peptides derived from the above-described four pre-erythrocytic-stage antigens, together with one control. Each of the four animals was immunized with one of the four molecules by using a mixture of peptides as described in Table 1. Immunizations were performed subcutaneously three times at intervals of 20 days. The final volume per injection was 500 μl containing 200 μg of each peptide. Six of the peptides were lipid-tailed peptides coupled with a palmitic acid at the carboxyl-terminal end using a lysine residue as a linker, which, on the basis of previous good immunogenicity results (3, 36), were injected in saline only, i.e., without an adjuvant. The remaining six peptides (without a lipidic component) were emulsified in Montanide ISA-51. All were produced by the stepwise solid-phase *tert*-butoxycarbonyl technique (39) in a 430A automated peptide synthesizer (Applied Biosystems, Foster City, Calif.) and checked for homogeneity by analytical reverse-phase high-pressure liquid chromatography and for identity by amino acid analysis (3).

Antibody production in response to peptide immunization. A high level of production of antibodies against 10 of the 12 peptides tested was observed. Sera collected from *Aotus* monkeys 15 and 210 days after the third immunization were tested in parallel by using standard enzyme-linked immunosorbent assay (ELISA) procedures described previously (6), except that rabbit anti-*Cebidae* monkey immunoglobulin G (IgG) (a gift of T. Fandeur, Institut Pasteur de Guyane, Cayenne, French Guiana), diluted 1/2,000, was used as the second antibody and revealed by peroxidase-conjugated anti-rabbit IgG (Biosys, Compiègne, France) at a dilution of 1/4,000.

As shown in Table 2, detectable antibodies against peptides LSA1-NR, LSA1-TER, and LSA1-REP were induced by the immunization scheme and, interestingly, found to increase thereafter, despite the fact that no further boosting had been performed. Only LSA1-J/Lipo did not induce antibodies; however, we observed in chimpanzees that the antibody response to this peptide was one which varied the most from one animal to another (3a). Responses to both SALSA peptides, which do not share cross-reactive epitopes (4), were elicited, and that against SALSA-2 was much stronger than that against SALSA-1. This is similar to what has been observed in immunized mice and chimpanzees, as well as in exposed African humans (4), confirming that SALSA-2 contains a potent B-cell epitope(s). Antibody responses to both STARP-R and STARP-M, two related peptides, were obtained; however, the response was strikingly stronger for STARP-M, which is a convergent combina-

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TABLE 1. Immunization scheme[a]

Protein (*Aotus* monkey)	Peptide or compound	Sequence
LSA1 (M21)	LSA1-J/Lipo	ERRAKEKLQEQQSDLEQRKADTKK K(Pam)-NH2[b]
	LSA1-NR	DTKKNLERKKEHGDILAEDLYGRLEIF
	LSA1-REP	LAKEKLQEQQSDLEQRLAKEKLQEQQSDLEQRLAKEKLQ
	LSA1-TER	NSRDSKEISIIEKTNRESITTNVEGRRDIHKGHEGHL
LSA3 (M23)	LSA3-NRI	DELFNELLNSVDVNGEVKENILEESQ
	LSA3-NRII/Lipo	LEESQVNDDIFNSLVKSVQQEQQHNV K(Pam)-NH2
	LSA3-RE	VESVAPSVEESVAPSVEESVAENVEESV
	LSA3-CT1/Lipo	LLSNIEEPKENIIDNLLNNI K(Pam)-NH2
SALSA (M4)	SALSA-1/Lipo	SAEKKDEKEASEQGEESEEKKENSQESA K(Pam)-NH2
	SALSA-2	NGKDDVKEEKKTNEKKDDGKTDKVQEKVLEKSPK
STARP (M48)	STARP-R/Lipo	STDNNNTKTISTDNNNTKTI K(Pam)-NH2
	STARP-M/Mixotope/Lipo	STDNNNTKTISTDNNNTNTI K(Pam)-NH2 L---T-NTIKA---S-IT-N ----D--D-NL------D-T -------K---------K-K
Adjuvant (V63)	Montanide ISA-51	

[a] *Aotus* monkeys were immunized with combinations of (i) lipopeptides (Lipo) injected in phosphate-buffered saline without an adjuvant at one location (pooled when there was more than one lipopeptide from each molecule) and (ii) pools of peptides emulsified with Montanide ISA-51 adjuvant (SEPPIC, Paris, France) injected on the same day at another site. STARP-M/Mixotope/Lipo consist of a mixed-epitope degenerated sequence as indicated above and described in reference 20.
[b] Peptide linked to palmitic acid (Pam) via a lysine residue.

torial library of peptides, or mixotope, obtained in a single synthesis by introducing degenerated sequences into the 10-amino-acid repeat region of the protein (see Table 1). This result is in keeping with epidemiological observations (21) and with previous studies using viral models in which the mixotope strategy was found to enhance immunogenicity (18, 23). The antibody responses to STARP peptides are also of interest because in vitro studies have indicated that they can significantly reduce sporozoite invasion in human hepatocytes and therefore may play a role in protection (20). Antibodies to three of the four LSA3 peptides, LSA3-CT1, LSA3 NR-II, and LSA3-RE, were produced, although at low titers only. These were nevertheless specific, since preimmunization sera were negative even when tested at 1:20, as were postimmunization samples tested with the control antigens RESA (47) and MSP3 (40). Although titers were low, they persisted for a long time, i.e., up to 7 months, after immunization. This lower immunogenicity of LSA3 peptides in the single *Aotus* monkey studied contrasts with the high titers induced in mice of various breeds and in outbred chimpanzees (3, 3a). It is noteworthy that we obtained strong antibody responses not only to repetitive motifs, as is frequently the case (31), but also to nonrepetitive regions of the molecules (e.g., LSA1-NR, LSA1-TER, and SALSA-2).

Production of T-cell responses. Remarkably, all of the 12 peptides derived from the four *P. falciparum* pre-erythrocytic molecules contained T-cell epitopes capable of inducing either proliferation, high gamma interferon, (IFN-γ) production, or, more frequently, both in *Aotus* lymphocytes (Fig. 1). In this case, production of IFN-γ has particular significance, since it is recognized as a major mechanism of defense against liver stage parasites (45). *Aotus* cells collected on day 0 (preimmunization) and 15 days after the third immunization were tested by lymphoproliferation with 10 μg of each peptide per ml as described elsewhere (26). IFN-γ concentrations in supernatants collected from triplicate wells on day 3 were assessed by a two-site capture ELISA (3, 4, 22) using a combination of anti-human IFN-γ monoclonal antibodies identified as able to react with *Aotus* IFN-γ. Each of the four LSA1 peptides contained T-cell determinants capable of stimulating IFN-γ production, and three of the four peptides induced a significant proliferative response. When tested 210 days postimmunization, a strong lymphoproliferation in response to two of the four peptides, LSA1-NR and LSA1-REP, was still detectable (data not shown). We found secretion of IFN-γ in response to each of the four LSA3 peptides and T-cell proliferation in response to two of the four peptides, LSA3-NRI and LSA3-

TABLE 2. Antibody responses[a]

Monkey	Antigen	ELISA titer on post-immunization day:	
		15	210
M21	LSA1-J	<100[b]	<100
	LSA1-NR	2,700	8,100
	LSA1-REP	100	300
	LSA1-TER	2,700	8,100
M23	LSA3-NRI	<100	<100
	LSA3-NRII	100	100
	LSA3-RE	100	100
	LSA3-CT1	100	100
M4	SALSA-1	100	100
	SALSA-2	2,700	8,100
M48	STARP-R	2,700	2,700
	STARP-M	24,300	8,100

[a] Titers determined in samples taken 15 and 210 days postimmunization correspond to the dilution of the test sera whose optical density at 450 nm was above the mean of control *Aotus* sera plus 2 standard deviations. Controls included (i) sera from 10 *Aotus* monkeys with no history of exposure to malaria, as well as preimmunization sera from the immunized monkeys, and (ii) peptides RESA and MSP3. The mean optical densities of the control *Aotus* sera against each of the peptides, used to define the cutoff values, ranged between 0.02 and 0.18.
[b] Negative at a dilution of 1:100.

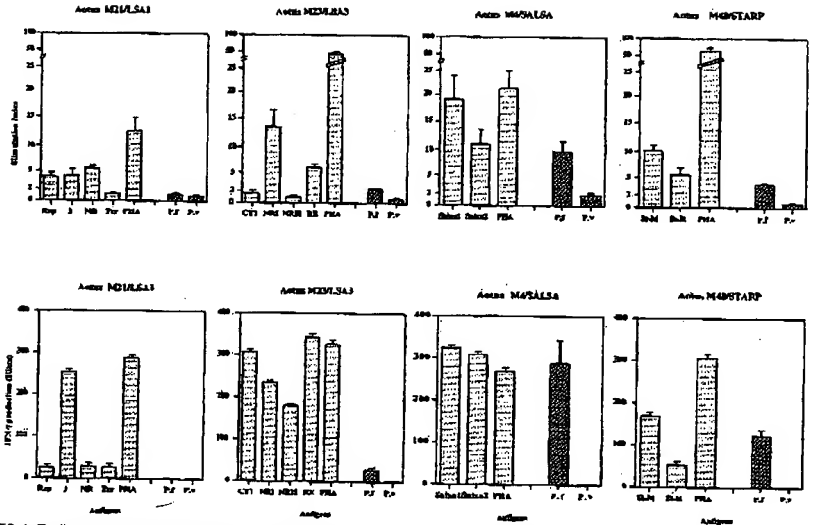

FIG. 1. T-cell proliferative responses and IFN-γ secretion. The stimulation index was calculated as the mean number of counts per minute of triplicate test cultures divided by the mean number of counts per minute of triplicate control cultures (nonstimulated cells) and was considered positive when >2. IFN-γ concentrations were calculated from a standard curve included in each plate and made by using culture medium containing known amounts of a standard IFN-γ (NIH-IFN-γ Gp23-901-530). Negative and positive controls (unstimulated cells and cells stimulated with phytohemagglutinin [PHA]) were included in each assay. Supernatants from human and *Aotus* phytohemagglutinin-stimulated blasts yielded similar values in the assay. Synthetic peptides and sonicated *P. falciparum* (P.f) sporozoite extracts (NF54 strain; a gift of W. Eling) were adjusted to a final protein concentration of 10 μg/ml. *P. vivax* (P.v) sporozoites (obtained from *Anopheles albimanus* mosquitoes fed on human blood samples) and noninfected *A. albimanus* mosquito salivary gland extracts were used as controls.

RE. This is reminiscent of studies with rodents in which LSA3-CTI/Lipo was capable of inducing a T-cell response that could only be revealed in terms of IFN-γ production (41a). The presence of T-cell epitopes for *Aotus* lymphocytes within both SALSA-1 and SALSA-2 was shown by the induction of both proliferative and IFN-γ responses to these peptides. Cellular responses were also positive with the two STARP peptides. They were also long lasting, as they remained detectable 210 days postimmunization (data not shown). As was the case for antibody production, both lymphoproliferation and IFN-γ production were slightly higher with the multiepitope peptide STARP-M. The specificity of the above-described responses was ascertained by negative results recorded for two control monkeys tested in parallel, as well as with preimmunization lymphocyte samples from immunized *Aotus* monkeys.

Relevance to native parasite proteins. Synthetic peptides may not always properly mimic the conformation of epitopes within the whole parasite protein. This question is of particular importance, as it can affect firstly the protective properties of the immune responses and secondly the ability of the parasite to boost it upon challenge. It was thus addressed at both the B- and T-cell levels. Antibody titers were determined by testing twofold serial dilutions in a "wet" indirect fluorescent-antibody test (IFAT) as previously described for sporozoites (15) and using Carnoy-fixed *P. falciparum* liver schizont sections as previously described for liver stage parasites (16).

Antibodies induced in the four immunized monkeys recognized the corresponding *P. falciparum* native proteins expressed on the sporozoite surface and/or in liver stage schizonts (Table 3). In agreement with our ELISA results, anti-STARP antibodies were the most reactive.

Aotus sera did not react with *P. vivax* sporozoites (titers of <1/20; data not shown), which do not share any homologous protein with the four *P. falciparum* antigens studied. The serum of *Aotus* monkey M21, immunized with LSA1 peptides, reacted specifically with liver schizonts but, as expected, not with

TABLE 3. IFAT determination of stage-specific antibody responses[a]

Monkey	Source of immunizing peptides	Sporozoites		Liver stage parasites	
		Day 15	Day 210	Day 15	Day 210
M21	LSA1	<20[b]	<20	100	100
M25	LSA3	100	100	100	100
M4	SALSA	100	100	<20	<20
M48	STARP	3,200	3,200	200	100

[a] Twofold serial dilutions in phosphate-buffered saline of serum from each monkey were tested in an IFAT, starting at 1:20. Each value is the reciprocal of the highest positive antibody dilution. Rabbit anti-*Saimiri* monkey IgG diluted 1:200 was employed, as in an ELISA, as the second antibody and detected with fluorescein-conjugated goat anti-rabbit IgG (heavy and light chains; Diagnostic Pasteur France) diluted at 1/100. Sera from 10 *Aotus* monkeys with no history of malaria were used as controls and found to be negative at a dilution of 1:20.

[b] Negative at a dilution of 1:20.

other parasite stages, thus confirming the strict liver stage expression of this molecule (22).

For technical reasons, T-cell responses can be assessed only with sporozoites (the output of in vitro and in vivo methods of liver stage *P. falciparum* production is too low). Nevertheless, it is interesting that an in-vitro challenge with *P. falciparum* sporozoites, but not with *P. vivax* (used as a control), could induce specific lymphoproliferation and IFN-γ production in cells from each of the three animals immunized with the three molecules expressed at the sporozoite stage, but not with LSA1, which is expressed only at the liver stage (Fig. 1). In our previous studies with chimpanzees (3a), each animal was immunized with LSA3 together with each of the other three molecules, and this impeded the precise determination of which pre-erythrocytic molecule was responsible for the T-cell proliferation observed with sporozoite extracts. The present results obtained with animals immunized with single molecules indicate that, in addition to LSA3, both synthetic peptides STARP and SALSA can also induce T-cell responses specific to native T-cell epitopes on sporozoites. Thus, the immune responses induced by artificial immunization were stage specific and relevant to native proteins.

Although the number of monkeys used in this study was small, the immune responses observed confirm the high immunogenicity of our molecules and stress the interest of the lipopeptide strategy. In recent years, synthetic lipopeptide technology has received more consideration for vaccine delivery (5, 13, 36, 49). Although the design of the experiment does not permit immunogenicity comparisons between identical peptides in adjuvants versus lipopeptides, the present results obtained with *Aotus* monkeys support previous indications (3). We tested six lipopeptides in *Aotus* monkeys, and, remarkably, all of them were able to induce high T-cell and/or humoral responses without an adjuvant. This contrasts with another study in which human immunodeficiency virus-derived lipopeptides were injected into macaques and the animals responded to this formulation only when it was mixed with incomplete Freund's adjuvant (5) and is in favor of the immunogenicity of the pre-erythrocytic molecules under study. We cannot exclude the possibility that the Montanide adjuvant injected with the peptides into the same animal, albeit at a different site, indirectly influenced the immunogenicity of the lipopeptides. However, it is noteworthy that the strongest immune responses in our experiments were generated in the *Aotus* monkey immunized with lipopeptides only, and the same has been previously observed in mice and chimpanzees (3, 3a).

Indeed, the results sound promising when compared to those of other trials performed with *Saimiri* and *Aotus* monkeys and antigens derived from *Plasmodium* organisms at various stages of the life cycle. These were done by using different antigen formulations and different adjuvants and therefore cannot be strictly compared. Nevertheless, it is striking that strong immune responses were obtained in the above studies only with powerful adjuvants, the majority of the studies relying on Freund's adjuvant (7, 19, 25, 27, 32, 41–43). Despite this fact, only 10% of the animals developed strong immune responses, 44% developed moderate responses, 35% developed weak responses, and 9% did not respond at all (1, 7, 9, 19, 25, 27, 32, 33, 41–43). In contrast, the substantial and long-lasting responses obtained by using lipopeptides without any adjuvant or a mixture of peptides with an adjuvant which can be used in humans compare favorably with those in previous studies with the same animal species.

The strategy employed to identify both the four antigens and the specific peptides may have particularly favored the selection of more immunogenic molecules (35). The screening process of an initial set of 120 clones encoding *P. falciparum* pre-erythrocytic molecules included several steps which involved the selection of immunodominant B- and T-cell epitopes. Strong B-cell epitopes were identified by screening with human antibodies obtained from different sources: (i) a set of 15 African human sera from areas of endemicity, (ii) antibodies affinity purified on each recombinant protein and tested upon sporozoites and liver stage parasites, and (iii) sera from "postimmune" individuals who had left the area of endemicity several years before to determine if the immune response were long lasting. It is our belief that this strategy favored the selection of more conserved and immunodominant antigens containing not only B-cell epitopes but also strong T-helper epitopes associated with them. The T-cell epitopes were selected by taking advantage of the observation that they are frequently localized in unstable regions close to regularly organized structures susceptible to proteolysis and therefore susceptible to being processed and associated with major histocompatibility complex molecules (3). Since it has been shown that T-cell epitopes could overlap and segregate within a relatively small area of a given molecule (2, 28, 37, 48), the synthesis of medium-size peptides (20 to 41 amino acids) was chosen to increase the chance of getting several T-cell epitopes recognized by various class II antigens in one given peptide.

We have found that the immunization of *Aotus* monkeys with the 12 peptides described above corroborated data about their antigenicity obtained with individuals exposed to malaria: among the 12 peptides, 11 were found to define B-cell epitopes in human populations with high antibody production, and all 12 defined T-cell epitopes with high prevalence to 9 of the 12 peptides (4, 12a, 21, 22). Moreover, the value of each of the four molecules was supported by the identification of numerous cytotoxic T-lymphocyte epitopes (3, 3a, 4, 29). Data obtained with *Aotus* monkeys therefore confirm the previous indications about the good antigenicity of these four molecules in various species (3, 3a, 4, 6a, 12a, 21, 22) and show that these medium-size peptides can associate with major histocompatibility complex class II antigens of the *Aotus* species as they do with those of humans (22).

So far, *Aotus* monkeys have been used mainly for assessment of the immunogenicity and protective efficacy of several blood stage antigens (7, 9, 10, 27, 32, 42) but not much for pre-erythrocytic vaccine development; hence, the reproducibility of this model, in parasitological terms, has yet to be defined. Nevertheless, preliminary studies conducted with *Aotus*, *Saimiri*, and *Cebus* monkeys have indicated that, in contrast to blood stage parasites, liver schizogony can be readily obtained with *P. falciparum* strains without the need for previous adaptation to these monkeys (11, 12, 13a, 14, 16). This, together with the good antigenicity and immunogenicity of the pre-erythrocytic-stage molecules described in this paper and the relevance of responses to native proteins, suggests that *Aotus* monkeys have potential for preclinical steps in the development of pre-erythrocytic-stage malaria vaccines.

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REFERENCES

1. Barr, P. J., K. M. Green, H. L. Gibson, I. C. Bathurst, I. A. Quakyi, and D. C. Kaslow. 1991. Recombinant PMS protein of *Plasmodium falciparum* elicits malaria transmission-blocking immunity in experimental animals. J. Exp. Med. 174:1203–1208.
2. Braunbarer, S., C. J. Wallace, A. E. Proudfoot, C. Bron, and G. Corradin. 1987. Multiple T cell antigenic determinants identified within a limited region of the horse cytochrome c molecule. Eur. J. Immunol 17:651–656.
3. BenMohamed, L., B. Gras-Masse, A. Tartar, P. Daubersies, K. Brahimi, M. Bossus, A. Thomas, and P. Druilhe. 1997. Lipopeptide immunization without adjuvant induces potent and long-lasting B, T helper, and cytotoxic T lymphocyte responses against a malaria liver stage antigen in mice and chimpanzees. Eur. J. Immunol 27:1242–1253.
3a. BenMohamed, L. et al. Submitted for publication.
4. Bottius, E., L. BenMohamed, K. Brahimi, H. Gras-Masse, J.-P. Lepers, L. Raharimalala, A. Aikawa, J. F. G. M. Meis, B. Sterenbregt, A. Tartar, A. Thomas, and P. Druilhe. 1996. A novel *Plasmodium falciparum* sporozoite and liver stage antigen (SALSA) defines major B, T helper, and CTL epitopes. J. Immunol. 156:2874–2884.
5. Bourgault, I., A. Chirat, A. Tartar, J. P. Levy, J. G. Guille, and A. Verer. 1994. Simian immunodeficiency virus as a model for vaccination against HIV: induction in *Aotus macaques* of GAG or NEF specific cytotoxic T lymphocytes by lipopeptides. J. Immunol. 152:2530–2537.
6. Brahimi, K., J.-L. Périgaud, M. Bossus, H. Gras, A. Tartar, and P. Druilhe. 1993. Fast immunopurification of small amounts of specific antibodies on peptides bound to ELISA plates. J. Immunol. Methods 162:69–75.
6a. Brasseur, P., et al. Submitted for publication.
7. Chang, S. P., S. E. Case, W. L. Gosnell, A. Hashimoto, K. J. Kramer, L. Q. Tam, C. Q. Hashiro, C. M. Nikaido, H. L. Gibson, C. T. Lee-Ng, P. J. Barr, B. T. Yokota, and G. S. N. Hui. 1996. A recombinant baculovirus 42-kilodalton C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 protects *Aotus* monkeys against malaria. Infect. Immun. 64:253–261.
8. Collins, W. E. 1992. South American monkeys in the development and testing of malarial vaccines. Mem. Inst. Oswaldo Cruz. Rio de J. 87:401–406.
9. Collins, W. E., R. F. Anders, I. Ruebusch, D. J. Kemp, G. C. Woodrow, G. H. Campbell, G. V. Brown, O. Irving, N. Gras, V. K. Filipski, R. L. Coppel, J. R. Brodersen, H. Thomas, D. Pye, J. C. Skinner, C. Wilson, P. S. Staniflt, and P. M. Procell. 1991. Immunization of owl monkeys with the ring-infected erythrocyte surface antigen of *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. 44:34–41.
10. Collins, W. E., G. G. Galland, J. S. Sullivan, and C. L. Morris. 1994. Selection of *Aotus* monkey model for testing *Plasmodium falciparum* blood-stage vaccine. Am. J. Trop. Med. Hyg. 51:224–232.
11. Collins, W. E., G. G. Galland, J. S. Sullivan, C. L. Morris, B. B. Richardson, and J. M. Roberts. 1996. The Santa Lucia strain of *Plasmodium falciparum* as a model for vaccine studies. I. Development in *Aotus lemurinus griseimembra*. Am. J. Trop. Med. Hyg. 54:372–379.
12. Collins, W. E., M. Warren, J. C. Skinner, W. Chin, and B. B. Richardson. 1977. Studies on the Santa Lucia (El Salvador) strain of *Plasmodium falciparum* in *Aotus trivirgatus* monkeys. J. Parasitol. 63:52–56.
12a. Daubersies, P., et al. Unpublished data.
13. Deres, K., H. Schild, K. Wiesmuller, G. Jung, and H. Rammensee. 1989. *In vivo* priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. Nature 342:561–564.
13a. Druilhe, P. Unpublished data.
14. Druilhe, P., and C. Marchand. 1989. From sporozoite to liver stages: the saga of the irradiated sporozoite vaccine. p. 39–48. *In* K. McAdam (ed.), New strategies in parasitology. Churchill Livingstone. Edinburgh, Scotland.
15. Druilhe, P., O. Pradier, J. P. Marc, F. Miltgen, D. Mazier, and D. Parent. 1986. Levels of antibodies to *Plasmodium falciparum* sporozoite surface antigens reflect malaria transmission rates and are persistent in the absence of reinfections. Infect. Immun. 53:393–397.
16. Druilhe, P., R. M. Pochis, F. Miltgen, L. Perrin, and M. Gentilini. 1984. Species- and stage-specific antigens in exoerythrocytic stages of *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. 33:336–341.
17. Edelman, R., S. L. Hoffman, J. R. Davis, N. Beier, M. B. Sztein, G. Losonsky, D. A. Herrington, M. A. Eddy, M. R. Hollingdale, D. M. Gordon, and D. F. Clyde. 1993. Long-term persistence of sterile immunity in a volunteer immunized with X-irradiated *Plasmodium falciparum* sporozoites. J. Infect. Dis. 168:1066–1070.
18. Estaquier, J., H. Gras-Masse, C. Boutillon, J. C. Ameisen, A. Capron, A. Tartar, and C. Auriault. 1994. The mixotope: a combinatorial peptide library as a T cell and B cell immunogen. Eur. J. Immunol. 24:2789–2795.
19. Etlinger, H. M., F. Caspers, H. Matile, H. J. Schoenfeld, D. Stueber, and B. Takacs. 1991. Ability of recombinant or native proteins to protect monkeys against heterologous challenge with *Plasmodium falciparum*. Infect. Immun. 59:3498–3503.
20. Fidock, D., V. Pasquetto, H. Gras, E. Badell, W. Eling, W. R. Ballou, J. Sedghid, A. Tartar, and P. Druilhe. 1997. *Plasmodium falciparum* sporozoite invasion is inhibited by naturally acquired or experimentally induced polyclonal antibodies to the STARP antigen. Eur. J. Immunol. 27:2502–2513.
21. Fidock, D. A., E. Bottius, K. Brahimi, I. I. M. Moelans, M. Aikawa, R. N. H. Konings, U. Certa, P. Olafsson, T. Kaidoh, A. Asavanich, C. Guerin-Marchand, and P. Druilhe. 1994. Cloning and characterization of a novel *Plasmodium falciparum* sporozoite surface antigen, STARP. Mol. Biochem. Parasitol. 64:219–232.
22. Fidock, D. A., H. Gras-Masse, J. P. Lepers, K. Brahimi, L. BenMohamed, S. Mellouk, C. Guerin-Marchand, A. Londono, L. Raharimalala, J. F. G. Meis, G. Langsley, C. Roussilhon, A. Tartar, and P. Druilhe. 1994. *Plasmodium falciparum* liver stage-specific antigen-1 is well conserved and contains potent B and T cell determinants. J. Immunol. 153:190–204.
23. Gras-Masse, H., J. C. Ameisen, B. C., F. Roussi, M. Bossus, B. Deprez, J. L. Neyrink, A. Capron, and A. Tartar. 1992. Synthetic vaccines and HTV-1 hypervariability: a "mixotope" approach. Pept. Res. 5:211–216.
24. Guérin-Marchand, C., P. Druilhe, B. Galey, A. Londono, J. Patarapotikul, R. Beaudoin, C. Dubeaux, A. Tartar, O. Mercereau-Puijalon, and G. Langsley. 1987. A liver stage-specific antigen of *Plasmodium falciparum* characterized by gene cloning. Nature 329:164–167.
25. Herrera, M., F. Rosero, S. Herrera, P. Caspers, D. Rotmann, F. Sinigaglia, and U. Certa. 1992. Protection against malaria in *Aotus* monkeys immunized with a recombinant blood-stage antigen fused to a universal T-cell epitope: correlation of serum gamma interferon levels with protection. Infect. Immun. 60:154–158.
26. Herrera, S., C. De Plata, J. M. Gonzalez, B. L. Perlaza, F. Betteas, G. Corradin, and M. Arevalo-Herrera. 1997. Antigenicity and immunogenicity of multiple antigen peptides (MAP) containing *P. vivax* CS epitopes in *Aotus* monkeys. Parasite Immunol. 19:161–170.
27. Herrera, S., M. A. Herrera, B. L. Perlaza, Y. Burki, F. Caspers, H. Döbeli, D. Rotmann, and U. Certa. 1990. Immunization of *Aotus* monkeys with *Plasmodium falciparum* blood-stage recombinant proteins. Proc. Natl. Acad. Sci. USA 87:4017–4021.
28. Higgins, J. A., C. J. Thorpe, J. D. Hayball, R. E. O'Hehir, and J. B. Lamb. 1994. Overlapping T-cell epitopes in the group I allergen of Dermatophagoides species restricted by HLA-DP and HLA-DR class II molecules. J. Allergy Clin. Immunol. 93:891–899.
29. Hill, A. V. S., J. Elvin, A. C. Willis, M. Aidoo, C. E. M. Allsopp, F. M. Gotch, X. M. Gao, M. Takiguchi, B. M. Greenwood, A. R. M. Townsend, A. J. McMichael, and H. C. Whittle. 1992. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. Nature 360:434–439.
30. Hoffman, S. L., E. D. Franke, M. R. Hollingdale, and P. Druilhe. 1996. Attacking the infected hepatocyte. p. 35–37. ASM Press, Washington, D.C.
31. Hollingdale, M. R., E. H. Nardin, S. Tharavanij, A. L. Schwartz, and R. S. Nussenzweig. 1984. Inhibition of entry of *Plasmodium falciparum* and *P. vivax* sporozoites into cultured cells: an *in vitro* assay of protective antibodies. J. Immunol. 132:909–913.
32. Inselburg, J., I. C. Bathurst, J. Kansopon, P. J. Barr, and R. Rossan. 1993. Protective immunity induced in *Aotus* monkeys by a recombinant SERA protein of *Plasmodium falciparum*: further studies using SERA 1 and MF75.2 adjuvant. Infect. Immun. 61:2048–2052.
33. Knapp, B., K. Hundt, B. Enders, and H. A. Küpper. 1992. Protection of *Aotus* monkeys from malaria infection by immunization with recombinant hybrid proteins. Infect. Immun. 60:2397–2401.
34. Londono, J. A., M. Sedegah, Y. Charoenvit, R. L. Beaudoin, and P. Druilhe. 1991. Antigenic analysis of *Plasmodium yoelii* liver stages by fluorescence antibody assays. Trop. Med. Parasitol. 42:381–385.
35. Marchand, C., and P. Druilhe. 1990. How to select *Plasmodium falciparum* pre-erythrocytic antigens in an expression library without defined probe. Bull. W. H. O. 68(Suppl.):158–164.
36. Martinon, F., H. Gras-Masse, C. Boutillon, F. Chirat, B. Deprez, J.-G. Guillet, E. Gomard, A. Tartar, and J.-P. Levy. 1992. Immunization of mice with lipopeptides bypasses the prerequisite for adjuvant. Immune response of BALB/c mice to human immunodeficiency virus envelope glycoprotein. J. Immunol. 149:3416–3422.
37. Meister, G. E., C. G. P. Roberts, J. A. Berzofsky, and A. S. De Groot. 1995. Two novel T cell epitope prediction algorithms based on MHC-binding motifs: comparison of predicted and published epitopes from *Mycobacterium tuberculosis* and HIV protein sequences. Vaccine 13:581–591.
38. Mellouk, S., F. Lunel, M. Sedegah, R. L. Beaudoin, and P. Druilhe. 1990. Protection against malaria induced by irradiated sporozoites. Lancet 335: 721.
39. Merrifield, R. B. 1963. Solid-phase peptide synthesis. The synthesis of a tetrapeptide. J. Am. Chem. Soc. 85:2149–2152.
40. Oeuvray, C. H. Bouharoun-Tayoun, H. Gras-Masse, E. Bottius, T. Kaidoh, M. Aikawa, M.-C. Filgueira, A. Tartar, and P. Druilhe. 1994. Merozoite surface protein-3: a malaria protein inducing antibodies that promote *Plasmodium falciparum* killing by cooperation with blood monocytes. Blood 84: 1594–1602.
41. Patarroyo, M. E., P. Romero, M. L. Torres, P. Clavijo, A. Martinez, R. Rodriguez, F. Guzman, and E. Cabezas. 1987. Induction of protective immunity against experimental infection with malaria using synthetic peptides. Nature 328:629–632.
41a. Perlaza, B. L., et al. Unpublished data.
42. Rodrigues, R., A. Moreno, F. Guzman, M. Calvo, and M. E. Patarroyo. 1990.

Studies of owl monkeys leading to the development of a synthetic vaccine against the asexual blood stages of *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. 43:339–354.
43. **Rochus, T. K. J., G. H. Campbell, A. Moreno, M. E. Patarroyo, and W. E. Collins.** 1990. Immunization of owl monkeys with a combination of *Plasmodium falciparum* asexual blood-stage synthetic peptide antigens. Am. J. Trop. Med. Hyg. 43:355–366.
44. **Schetter, L. F., and A. F. Azad.** 1995. Maintenance of protective immunity against malaria by persistent hepatic parasites derived from irradiated sporozoites. Proc. Natl. Acad. Sci. USA 92:4066–4068.
45. **Schofield, L., A. Ferreira, R. Altszuler, V. Nussenzweig, and R. S. Nussenzweig.** 1987. Interferon-γ inhibits intrahepatocytic development of malaria parasites *in vitro*. J. Immunol. 139:2020–2025.
46. **Sturchler, A. L. A. Wieger, E. Castellano, and R. Linden.** 1990. Survival and antigenic profile of irradiated malarial sporozoites in infected liver cells. Infect. Immun. 58:2834–2839.
47. **Troye-Blomberg, M., E. M. Riley, H. Perlmann, G. Andersson, A. Larsson, R. W. Snow, S. J. Allen, R. A. Houghten, O. Olerup, B. M. Greenwood, and P. Perlmann.** 1989. T and B cell responses of *Plasmodium falciparum* malaria-immune individuals to synthetic peptides corresponding to sequences in different regions of the *P. falciparum* antigen Pf155/RESA. J. Immunol. 143: 3043–3048.
48. **van Binnendijk, R. S., J. P. M. Versteeg-van Oosten, M. C. M. Poelen, H. F. Breggike, F. Boogerhout, A. D. M. E. Osterhaus, and F. G. C. M. Uytdehaag.** 1993. Human HLA class I- and HLA class II-restricted cloned cytotoxic T lymphocytes identify a cluster of epitopes on the measles virus fusion protein. J. Virol. 67:2276–2284.
49. **Vitiello, A., G. Ishioka, H. M. Grey, R. Rose, P. Farnsos, R. LaFond, L. Yuan, F. V. Chisari, J. Furze, R. Bortholomeyez, and R. W. Chesnut.** 1995. Development of a lipopeptide-based therapeutic vaccine to treat chronic HBV infection. Induction of a primary cytotoxic T lymphocyte response in humans. J. Clin. Invest. 95:341–349.

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Immunogenicity of four Plasmodium falciparum preerythrocytic antigens in Aotus lemurinus monkeys.

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Aotus lemurinus monkeys were immunized with pools of either lipid-tailed peptides in in PBS or peptides in Montanide ISA-51, all derived from four Plasmodium falciparum erythrocytic antigens, namely, LSA1, LSA3, SALSA, and STARP. These formulations well tolerated. Their immunogenicity was demonstrated by the induction of both B- an cell responses to most of the peptides studied (of the 12, 10 induced antibody productic induced T-cell proliferative responses, and all 12 induced gamma interferon secretion). Immune responses proved to be long lasting, since some were still detectable 210 days immunization. Of particular importance is the fact that B- and T-cell responses elicited this way by synthetic peptides were specific for native parasite proteins on P. falciparu sporozoites and liver stage parasites.

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